REMARKS

Applicants hereby cancel originally filed claims 1-26, and substitute therefore the new claims 27-64, which should be in condition for allowance as presented. The correspondence between the new claims and those of Amendment B of the parent application (allowed) as further amended is as follows:

New claim #s	Amendment B claim #s (same as original filed claim #s)
27-32	1-6 (allowed in parent)
33-36	
37-42	7-12 (allowed in parent)
43-46	
47-53	13-19 (allowed in parent)
54-57	
58-64	20-26 (allowed in parent)

The present amendments provide for subject matter supported in the specification and provide the full scope of the invention as conceived by the inventors, and in view of the knowledge and ordinary skill in the art at the time of filing. Relative to the presently allowed claims of the parent application, the new independent claims are not limited to "amplification-mediated displacement of the CpG-specific probe," and thus allow for explicit recitation of a broader range of CpG-specific probe types in the dependent claims. Essentially, the amendments are as follows:

First, the detection step (c) of the independent method claims (and corresponding "kit" elements) now recite detecting the methylated nucleic acid based on "an amplification-mediated, or amplification product-mediated change in a property of the CpG-specific probe, or in a property thereof in relation to another probe or primer," in place of "amplification-mediated displacement of the CpG-specific probe."

Second, new dependent claims recite FRET probes, LightCycler™-type hybridization probes, molecular beacon-type probes, and scorpion-type primers (comprising probes) in addition to dual-labeled TaqMan™-type probes.

Thus, the new dependent claims explicitly encompass the broader enabled range of CpG-specific probe types, consistent with the independent claims not being unnecessarily limited to "amplification-mediated displacement of the CpG-specific probe."

Support for these claims amendments is found in the originally-filed specification at, *inter alia*, page 8, lines 18-20, page 15, lines 24-26, and page 16, lines 7-19.

No new matter has been added.

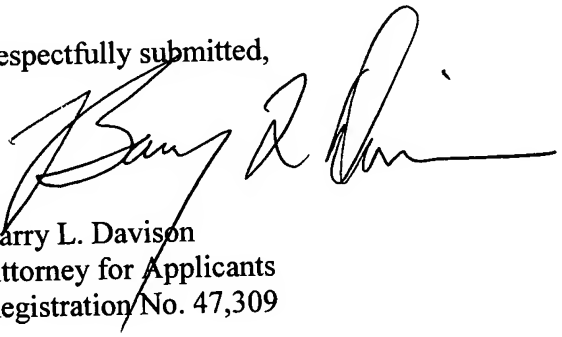
Formalities

Applicants, for clarity and in conformity with the underlying prosecution history, have amended the Brief Description of the Drawings in the specification by providing "clean" substitute paragraphs and corresponding "marked-up" paragraphs, such that "Figure 5" now reads as "Figures 5A and 5B," and "Figure 6" now reads as "Figures 6A-6C." Additionally, a paragraph entitled "Cross-reference to Related Applications" has been inserted into the specification at page 1.

Applicants respectfully request consideration and allowance of all pending new claims 27-64 of the present Continuation Application to provide for completion of claims supported by the original specification filed on 14 May 1999. The new claims 27-64 provide for subject matter supported in the specification and providing the full scope of the invention as conceived by the inventors, and in view of the knowledge and ordinary skill in the art at the time of filing.

No new matter has been added.

Respectfully submitted,



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APPENDIX A

("marked up" claims, corresponding to those prior allowed claims (*i.e.*, **as of Amendment B of the parent prosecution history**) that have been amended)

1 (new claim 27). A method for detecting cytosine methylation and methylated CpG islands within a genomic sample of DNA comprising:

(a) contacting a genomic sample of DNA with a modifying agent that modifies unmethylated cytosine to produce a converted nucleic acid;

(b) amplifying the converted nucleic acid by means of oligonucleotide primers in the presence of one or a plurality of specific oligonucleotide probes, wherein one or a plurality of the oligonucleotide primers or the specific probe(s) are capable of distinguishing between unmethylated and methylated nucleic acid, with the proviso that at least one oligonucleotide probe is a CpG-specific probe capable of distinguishing between unmethylated and methylated nucleic acid; and

(c) detecting the methylated nucleic acid based on an amplification-mediated, or amplification product-mediated change in a property of the CpG-specific probe, or in a property thereof in relation to another probe or primer [amplification-mediated displacement of the CpG-specific probe].

7 (new claim 38). A method for detecting a methylated CpG-containing nucleic acid comprising:

(a) contacting a nucleic acid-containing sample with a modifying agent that modifies unmethylated cytosine to produce a converted nucleic acid;

(b) amplifying the converted nucleic acid in the sample by means of oligonucleotide primers in the presence of a CpG-specific oligonucleotide probe, wherein the CpG-specific probe, but not the primers, distinguishes between modified unmethylated and methylated nucleic acid; and

(c) detecting the methylated nucleic acid based upon an amplification-mediated, or amplification product-mediated change in a property of the CpG-specific probe, or in a property thereof in relation to another probe or primer [an amplification-mediated displacement of the CpG-specific probe].

11 (new claim 42). The method of claim 38 [7] wherein the probe further comprises one or a plurality of fluorescence label moieties [detection method is by means of a measurement of a fluorescence signal based on amplification-mediated displacement of the CpG-specific probe].

14 (new claim 50). A method for detecting a methylated CpG-containing nucleic acid comprising:

(a) contacting a nucleic acid-containing sample with a modifying agent that modifies

unmethylated cytosine to produce a converted nucleic acid;

(b) amplifying the converted nucleic acid in the sample by means of oligonucleotide primers in the presence of a CpG-specific oligonucleotide probe, wherein both the primers and the CpG-specific probe distinguish between modified unmethylated and methylated nucleic acid; and

(c) detecting the methylated nucleic acid based on an amplification-mediated, or amplification product-mediated change in a property of the CpG-specific probe, or in a property thereof in relation to another probe or primer [amplification-mediated displacement of the CpG-specific probe].

18 (new claim 54). The method of claim 50 [14], wherein the probe further comprises one or a plurality of fluorescence label moieties [detection method comprises measuring a fluorescence signal based on amplification-mediated displacement of the CpG-specific probe].

20 (new claim 61). A methylation detection kit useful for the detection of a methylated CpG-containing nucleic acid comprising a carrier means being compartmentalized to receive in close confinement therein one or more containers comprising:

(i) [a first container containing] a modifying agent that modifies unmethylated cytosine to produce a converted nucleic acid;

(ii) [a second container containing] primers for amplification of the converted nucleic acid;

(iii) [a third container containing] primers for the amplification of control unmodified nucleic acid; and

(iv) [a fourth container containing] a [specific oligonucleotide] CpG-specific probe the detection of which is based on an amplification-mediated, or amplification product-mediated change in a property of the CpG-specific probe, or in a property thereof in relation to another probe or primer [amplification-mediated displacement], wherein the [specific oligonucleotide probe is a] CpG-specific [oligonucleotide] probe [that] distinguishes between modified unmethylated and methylated nucleic acid, and wherein the primers each may or may not distinguish between unmethylated and methylated nucleic acid.

23 (new claim 64). The kit of claim 20, wherein the CpG-specific [oligonucleotide] probe, but not the primers for amplification of the converted nucleic acid, distinguishes between modified unmethylated and methylated nucleic acid.

24 (new claim 65). The kit of claim 61 [20], wherein both the CpG-specific [oligonucleotide] probe and the primers for amplification of the converted nucleic acid, distinguish between modified unmethylated and methylated nucleic acid.

25 (new claim 66). The kit of claim 61 [20], wherein the CpG-specific probe further comprises one or a plurality of fluorescence label moieties [a fluorescent moiety linked to an oligonucleotide base directly or through a linker moiety].

26 (new claim 67). The kit of claim 66 [20], wherein the CpG-specific probe is a [specific] FRET probe, a LightCycler™-type hybridization probe, a dual-labeled TaqMan™ probe, or a molecular beacon-type probe.

APPENDIX B

("marked up" paragraphs, showing applicants' present amendments to originally-submitted text)

[Figure 5] Figures 5A and 5B [shows] show a comparison of the inventive assay to a conventional COBRA assay. Figure 5A (Panel A) [Panel A] shows a COBRA gel used to determine the level of DNA methylation at the *ESR1* locus in DNAs of known methylation status (sperm, unmethylated) and HCT116 (methylated). The relative amounts of the cleaved products are indicated below the gel. A 56-bp fragment represents DNA molecules in which the *Taq*I site proximal to the hybridization probe is methylated in the original genomic DNA. The 86-bp fragment represents DNA molecules in which the proximal *Taq*I site is unmethylated and the distal site is methylated. Figure 5B (Panel B) [Panel B] summarizes the COBRA results and compares them to results obtained with the methylated and unmethylated version of the inventive assay process. The results are expressed as ratios between the methylation-specific reactions and a control reaction. For the bisulfite-treated samples, the control reaction was a *MYOD1* assay as described in Example 1. For the untreated samples, the *ACTB* primers described for the RT-PCR reactions were used as a control to verify the input of unconverted DNA samples. (The *ACTB* primers do not span an intron). "No PCR" indicates that no PCR product was obtained on unconverted genomic DNA with COBRA primers designed to amplify bisulfite-converted DNA sequences.

[Figure 6] Figures 6A-6C [illustrates] illustrate a determination of the specificity of the oligonucleotides. Eight different combinations of forward primer, probe and reverse primer were tested on DNA samples with known methylation or lack of methylation at the *ESR1* locus. Figure 6A (Panel A) [Panel A] shows the nomenclature used for the combinations of the *ESR1* oligos. "U" refers to the oligo sequence that anneals with bisulfite-converted unmethylated DNA, while "M" refers to the methylated version. Position 1 indicates the forward PCR primer, position 2 the probe, and position 3 the reverse primer. The combinations used for the eight reactions are shown below each pair of bars, representing duplicate experiments. The results are expressed as ratios between the *ESR1* values and the *MYOD1* control values. Figure 6B (Panel B) [Panel B] represents an analysis of human sperm DNA. Figure 6C (Panel C) [Panel C] represents an analysis of DNA obtained from the human colorectal cancer cell line HCT116.